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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 041701. Fonofos (Dyfonate®). Draft Toxicology Chapter For Reregistration Eligibility

PC Code 041701
Tox. Chem. No. 454B
Project No. D229323

TO: John Redden, Chemical Manager
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THRU: Roger L. Gardner, Section Head
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Roger Gardner 8/30/96 KB 9/3/96

Fonofos was originally scheduled for a Reregistration Eligibility Decision (RED) document during the third quarter of 1996. At that time, the Toxicology Branch (TB-I) was requested to review the available toxicology studies on this chemical and write the toxicology chapter for the RED package. TB-I prepared the toxicology chapter only to find out that the Registrant for fonofos had voluntarily cancelled reregistration. Nevertheless, TB-I has decided to submit the chapter for possible future reference.

Attached to this cover memorandum is a draft of the Toxicology Chapter for the RED for fonofos. The Health Effects Division (HED) RfD Peer Review Committee met to discuss fonofos on August 12, 1993 and on June 20, 1996 and the Toxicology Endpoint Selection Committee met on August 6, 1996 to select endpoints for acute dietary, short term, intermediate term and chronic occupational or residential exposures.

FONOFOS:

TOXICOLOGY CHAPTER FOR RED

Barcode No. **D229323**
Rereg. Case No. **0105**
CAS No.: **944-22-9**

Submission No. **S497847**
Tox. Chem. No. **454B**
P.C. Code No. **041701**

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B. HUMAN HEALTH ASSESSMENT

1. Hazard Assessment

The toxicological data base for fonofos is not adequate to support reregistration eligibility as a food use pesticide. There are three major data gaps, a 21-day dermal study in the rat on the technical material (82-2), a multigeneration reproduction study (83-4) and a developmental neurotoxicity study (83-6).

a. Acute Toxicity

The acute toxicity data for fonofos are summarized in Table 1. All the acute studies are acceptable for regulatory purposes.

Table 1. Acute Toxicity Values for Fonofos

Guideline #	MRID No.	Study	Category
81-1	00078777	LD_{50} males: 24.5 mg/kg LD_{50} females: 10.8 mg/kg	I
81-2	00078777	LD_{50} both sexes: 159 mg/kg	I
81-3	41935901	LC_{50} males: 51.0 µg/l LC_{50} females: 17.9 µg/l	I
81-4	00078777	Mean score of 0.33 at 24 hours: non-irritating.	III
81-5	00078777	No irritation for 4/6 animals at 72 hours. Two animals died at 24 hours.	IV
81-6	42842601	A weak to mild sensitizer under conditions of study.	N/A

Acute oral toxicity studies in male and female rats indicated that oral exposure to fonofos induces clinical signs of toxicity that are typical of cholinesterase inhibitors. In males at the LD_{50} level, these signs included depression, tremors, copious salivation, diarrhea, bulging eyes, lacrimation, labored breathing and wet yellow stains around the ano-genital region. By day 6, these symptoms had disappeared. Any deaths occurred within 6 hours after dosing. In females at the LD_{50} level, depression, tremors, shallow breathing, blood-like stains around the facial area and yellow stains around the ano-genital region were observed. These signs disappeared by day 4. Deaths occurred within 22 hours.

An acute dermal toxicity study in rabbits indicated that dermal exposure to fonofos induces similar clinical signs of toxicity. At 200 mg/kg, depression, tremors, salivation,

diarrhea, rapid breathing and constricted pupils were observed in both sexes. These symptoms disappeared by day 4. Any deaths occurred within 5 hours. Necropsy of the animals which died showed red and irritated stomachs, darkened lungs and pale livers. Although only 3 or 4 rabbits/sex were tested/dose level, the study was considered acceptable because the results were consistent with the other acute toxicity studies.

An acute inhalation toxicity study was conducted on rats using a 4 hour exposure. The median lethal concentration was based on the atmospheric concentrations achieved in the study. Clinical signs of toxicity and cholinesterase inhibition were evident and were consistent with combination of neurological and irritancy effects which are typical of those seen following exposure to organophosphorus compounds.

In the primary dermal irritation study with rabbits, 0.05 ml was given as a dose instead of the required dose of 0.5 ml/site (0.2 ml/animal or 100 mg/kg) because in a previous study using 0.5 ml of 93% technical fonofos with Aliquot 335, all the animals had died. One hundred mg/kg had killed 2/6 animals in the acute dermal study.

In the primary eye irritation study, 0.01 ml was tested because in other eye irritation studies with technical dyfonate, all the animals died with a dose of 0.1 ml with no irritation. No rabbits died in this study. At 24 hours, 1/6 rabbits with unwashed eyes had a score of 2. This score was 0 by 48 hours. No other rabbits, either unwashed (6 animals) or washed (3 animals) had any reaction at any time. The mean score at 24 hours was 0.33. This corresponds to a rating of non-irritating. However, since one rabbit had an effect at 24 hours, this places the chemical in Toxicity Category III.

In the dermal sensitization study conducted with guinea pigs, a version of the maximisation test of Magnusson and Kligman was used. Formaldehyde was used as the positive control and provided an appropriate positive response.

b. Neurotoxicity

Technical fonofos (94.2%) was tested in an acute delayed neurotoxicity study in hens (Gallus gallus domesticus) by gavage at a constant dose volume of 10 ml/kg. A dose level of 143 mg/kg was selected for neurotoxicity study based on the results of a range finding study and an acute LD_{50} study. Two groups of fonofos treated birds were used because most of the birds died in the first group. In the second group, atropine was injected both prior to and after dosing instead of just prior to dosing. A vehicle control and a positive control (500 mg/kg tri-ortho-cresyl phosphate (TOCP)) were tested concurrently. The acute LD_{50} value was calculated to be 143 mg/kg (120-183 mg/kg). In

the neurotoxicity study, the fonofos treated birds displayed clinical signs of toxicity (unsteadiness, inability to stand and subdued behavior) which disappeared by day 6 in surviving birds. There was no clinical evidence of delayed neurotoxicity (ataxia) in the treated birds and the levels of neurotoxic esterase (NTE) for these birds were similar to vehicle controls. The positive control birds gave a weak positive response for delayed ataxia, but displayed a strong reduction in NTE. There was a 51% reduction in acetyl cholinesterase levels in the brain for the fonofos treated birds when compared to vehicle controls. Trace axonal degeneration was observed in the spinal cord and peripheral nerves of 5/6 of the vehicle controls. In the positive control birds, 4/6 birds showed minimal axonal degeneration in the spinal cord and 1/6 in the proximal sciatic nerve. In addition, trace axonal degeneration was observed in the cerebellum of 3/6 birds. In the fonofos treated birds, trace axonal degeneration was observed in the spinal cord and the peripheral nerves of 6 birds and in the cerebellum of 1 bird. In one bird, significant axonal degeneration (moderate or marked) was observed in the distal sciatic and tibial nerves on the right side only. In light of the facts that there was no clinical evidence of acute delayed neurotoxicity, there was no evidence of a decrease in NTE activity in animals treated with fonofos in this study, the positive controls displayed an unusually weak response and there was no evidence of delayed neurotoxicity in the 90-day study, this finding is considered to be an equivocal response. Therefore, fonofos is considered to have induced an equivocal response in the acute delayed neurotoxicity study (Core Guideline; 81-7; MRID 43161301).

Fonofos (Dyfonate®, 94.6%) was tested in an acute mammalian neurotoxicity study in Alpk:APfSD rats at the following dose levels: 0, 2, 4 or 7 mg/kg. Ten animals/sex were tested at each dose level. The doses were administered once in corn oil by gavage at 1 ml/100 g body weight. The following parameters were observed and measured: clinical signs of toxicity, body weights, food consumption, functional observational battery, motor activity, brain measurements and neuropathology. At 7 mg/kg, one female displayed reduced foot withdrawal reflex, shaking, signs of urinary incontinence, tip toe gait and upward curvature of the spine 6 hours after dosing. Recovery in this animal was observed by 24 hours. The NOEL is 4 mg/kg and the LOEL is 7 mg/kg based on clinical signs of toxicity. Appropriate positive control data were provided which indicated that the test system is capable of detecting neurotoxicological effects. In addition, results from a preliminary range-finding study were submitted which indicated that the dose levels selected were appropriate (Core Guideline; 81-8; MRID's 42777801 and 43030101).

Technical Dyfonate® was administered orally to adult hens for 90 days at 2, 4 and 8 mg/kg/day. Control groups were either untreated or given corn oil. The positive control group was

administered tri-o-cresyl phosphate (TOCP). No evidence of delayed neurotoxicity was observed in any of the Dyfonate^{R}-treated hens, whereas the positive controls displayed marked evidence of delayed neurotoxicity in addition to progressive loss of body weight, inhibition of plasma cholinesterase, impaired egg production and death. The Dyfonate^{R}-treated animals exhibited significant weight loss in the high dose group, clinical signs of toxicity in the mid- and high dose groups (possibly the low dose group), inhibition of plasma cholinesterase in all dose groups and impaired egg production in all dose groups. The NOEL for inhibition of plasma cholinesterase is < 2.0 mg/kg/day (LDT) and the NOEL for other acute neurotoxic effects is < 2.0 mg/kg/day (Core Minimum; 82-5; MRID 40150120).

Fonofos (Dyfonate®) was tested in a subchronic mammalian neurotoxicity feeding study in Alpk:APfSD rats at the following dose levels for 90 days: 0, 15, 50 or 125/150 ppm (0, 0.75, 2.5, or 6.25/7.5 mg/kg/day). The highest dose level was changed from 125 ppm to 150 ppm at week 5. Twelve rats/sex were tested at each dose level. The following parameters were observed and measured: clinical signs of toxicity, body weights, food consumption, functional observational battery, motor activity, brain measurements, cholinesterase activity and neuropathology. Six animals/sex in each group were designated for terminal neuropathology, although only the high dose group and controls were ultimately examined. At 15 ppm, statistically significant decreases in erythrocyte cholinesterase activity (both sexes) and in plasma cholinesterase activity (females) were observed. At 50 ppm and above, statistically significant decreases in cholinesterase activity were observed in both sexes for all 3 parameters. At 125/150 ppm, treatment related clinical signs were observed in females. These included upward curvature of the spine, tiptoe gait, signs of urinary incontinence, pinched in sides, reduced splay reflex, splayed gait, eye bulging and shaking. In addition to these, decreases in the motor activity observations were noted for females. There were no microscopic indications of neurotoxicity. The NOEL is 50 ppm and the LEL is 125/150 ppm based on clinical signs of toxicity and on decreases in motor activity. The NOEL for cholinesterase inhibition is 15 ppm and the LEL is 50 ppm based on decreases in cholinesterase activity in all 3 parameters at 50 ppm (Core Guideline; 82-7; MRID's 42792601 and 43030101).

c. Subchronic Toxicity

No acceptable subchronic feeding studies are available in either the rodent or nonrodent. The regulatory requirement for a subchronic feeding study in the rodent is satisfied by an acceptable chronic feeding study in the rat (MRID 40617901) and the regulatory requirement for a subchronic feeding study in the nonrodent is satisfied by an acceptable chronic oral study in the dog (MRID 43914601). A 21-day dermal study (82-2) is required.

d. Chronic Feeding

Technical fonofos (94%) was administered in the diet to groups of 50 Sprague-Dawley CD rats/sex/dose for 24 months at levels of 0, 4, 15, or 60 ppm and groups of 20/sex at 120 ppm for 12 months. The mean compound intake (averaged across sexes) was approximately 0.17, 0.65, 2.6 and 6.6 mg/kg/day at 4, 15, 60 or 120 ppm, respectively. Survival was not affected by dosing. Mean body weights and weight gains were significantly ($p < 0.05$ or < 0.01) depressed in females but not males receiving 120 ppm; over 52 weeks, weight gain was 21% lower in these females than in controls. Serum cholinesterase activity was significantly ($p < 0.01$) depressed in both sexes receiving 120 ppm; depression compared to controls at 3, 6, and 12 months was 38, 38, and 33% in males and 59, 56, and 54% in females. Erythrocyte cholinesterase activity was not affected in males or females receiving 120 ppm, but brain cholinesterase was depressed 35% in females ($p < 0.01$). At 60 ppm, serum cholinesterase activity was moderately depressed in males but more markedly depressed in females; erythrocyte cholinesterase activity was depressed only at 18 and 24 months in both sexes receiving 60 ppm ($p < 0.01$). Brain cholinesterase was not affected in males but was depressed 35% and 14% at 12 months in females receiving 120 and 60 ppm, respectively. No effects of dosing were observed on clinical laboratory findings (other than cholinesterase), or on organ weights or gross necropsy. The systemic NOEL is 2.6 mg/kg/day and the LEL is 6.6 mg/kg/day based on decreases in body weight and body weight gain. The NOEL for cholinesterase inhibition is 0.65 mg/kg/day and the LEL is 2.6 mg/kg/day based on inhibition of cholinesterase activity (brain, serum and erythrocyte) (Core Minimum; 83-5; MRID 40617901).

In a chronic toxicity study fonofos (94.6% a.i.) was administered to groups of 4 beagle dogs/sex/dose by capsule at dose levels of 0, 0.2, 0.4 or 1.75 mg/kg/day in corn oil for a period of at least one year. Observations and measurements included the usual required parameters plus cholinesterase activities (ChE). Additional (satellite) groups of 2 males and 2 females were dosed with either 0 or 1.75 mg fonofos/kg/day for 4 weeks. For these dogs, clinical observations were recorded as well as bodyweights, food consumption and ChE activity (including brain).

At 0.2 mg/kg/day, minimal sporadic plasma cholinesterase inhibition was observed in both sexes (7-13%; 20% only once at 52 weeks in females). At 1.0 mg/kg/day, there were increases in alkaline phosphatase levels (130-194% of control values) and inhibition of erythrocyte (51% in males, 53% in females) and plasma cholinesterase (50% in both sexes) activities. At 1.75 mg/kg/day, there were clinical signs of toxicity in one animal, decreases in serum albumin and total protein levels, increases in alkaline phosphatase levels (up to 217%), inhibition of

erythrocyte (62% in males, 63% in females), plasma (57% in males, 58% in females) and possibly brain (20% in females) cholinesterase activities and increases in absolute liver weights in males (18.5%). One female dosed with 2.0 mg/kg/day for 3 days developed clinical signs and intussusception of the terminal ileum, possibly due to uncontrolled peristaltic movement following substantial depression of cholinesterase activity. The NOEL is 0.2 mg/kg/day. The NOEL is considered to be a borderline NOEL/LOEL because there was minimal plasma cholinesterase inhibition at 0.2 mg/kg/day which was generally weak and was not consistent. The LOEL, 1.0 mg/kg/day is based on plasma and erythrocyte cholinesterase inhibition and increases in alkaline phosphatase levels at 1.0 mg/kg/day and above, and clinical signs of toxicity, decreases in selected blood chemistry values, increases in liver weights and histologic changes in the ileum at 1.75 mg/kg/day (Acceptable; 83-1b; MRID 43914601).

Technical fonofos (99.5% and 99.8-99.9%) was tested in a chronic feeding study in male and female beagle dogs at 0, 16(8.0), 60 and 240 ppm for 2 years (0, 0.4(0.2), 1.5, 6 mg/kg/day). Four dogs/sex/dose were tested. In the high dose female group, one dog was replaced during week 6 of the study. Double portions of the diet (both treated and control) were fed on Saturdays with no food offered on Sundays until the 85th week of the study. Thereafter, single portions were fed daily 7 days/week. At 0.4 mg/kg/day and above, decreases in erythrocyte cholinesterase levels were observed (61% of controls). Therefore, at week 14, the lowest dose level was decreased to 0.2 mg/kg/day. Plasma cholinesterase was decreased at 1.5 mg/kg/day and above (as low as 43% of controls for males and 52% for females). There were no effects on brain cholinesterase at any dose level. For systemic toxicity, at 1.5 mg/kg/day, clinical signs (conjunctival hyperemia and lacrimation), liver weight increases and slight body weight decreases (83-100% of controls in males) were observed. At 6 mg/kg/day, deaths, clinical signs (emesis, diarrhea, soft stools, alopecia, increased lacrimal, nasal and salivary excretions, nervous behavior, tremors and conjunctival and gingival hyperemia), decreases in body weight (as low as 71% of controls in males), increases in serum alkaline phosphatase (over 615% of controls in males and 283% in females), liver effects (increases in organ weights (108%) and increased numbers of binucleated hepatocytes, increased hepatic cell pigmentation and some homogeneity and eosinophilia of hepatocellular cytoplasms) and acute tissue congestion were observed. The cholinesterase NOEL is 0.2 mg/kg/day and the LOEL is 0.4 mg/kg/day. The systemic NOEL is 0.4(0.2) mg/kg/day and the LOEL is 1.5 mg/kg/day. There were major deficiencies with this study. These include: an unusual feeding pattern; no information on the frequency of diet preparation, storage, stability of the test chemical in the diet, homogeneity of mixing or concentration analyses; in the high dose group, a replacement dog was started 6 weeks into the study and did not appear to be

kept an extra 6 weeks at the other end of the study; no electrolytes were measured for the clinical chemistry analyses; the microscopic examinations were incomplete and statistical calculations were not conducted (Core Supplementary; 83-1; MRID 00082233).

e. Carcinogenicity

Technical fonofos (94%) was administered in the diet to groups of 50 Sprague-Dawley CD rats/sex/dose for 24 months at levels of 0, 4, 15, or 60 ppm and groups of 20/sex at 120 ppm for 12 months. The mean compound intake (averaged across sexes) was approximately 0.17, 0.65, 2.6 and 6.6 mg/kg/day at 4, 15, 60 or 120 ppm, respectively. Survival was not affected by dosing. No effects of dosing were observed on neoplastic findings (Core Minimum; 83-5; MRID 40617901; for other effects, see chronic feeding study in the rat in the chronic feeding section).

Technical fonofos (94%) was administered in the diet to groups of 50 CD-1 mice/sex/dose for 18 months at levels of 0, 5, 25 or 100 ppm (males: 0, 1, 3, or 12 mg/kg/day; females: 0, 1, 4 and 15 mg/kg/day). Ten extra mice/sex/dose were scheduled for an interim sacrifice. Fonofos was not carcinogenic under the conditions of the study. Serum cholinesterase activity was reduced in the mid-dose males at 12 months (53 and 20% of controls, respectively) and in the high dose males and females at 12 (20% and 32%) and 18 months (35% and 43% for males and females, respectively). Brain cholinesterase activity was depressed in high dose males at 12 (42%) and 18 months (62% of controls) and in the mid- and high dose females at 18 months (85 and 57% of controls, respectively). Inhibition of erythrocyte activity was noted in the high dose males and females at 18 months (51 and 37%, respectively). At 12 mg/kg/day (15 for females), raised foci, masses, thickening, hyperplasia and hypertrophy of the duodenum (males) and slight reductions in body weight (95% of controls for males, $p < 0.05$ at most of the weekly intervals during the first 13 weeks), body weight gain and food consumption (males) were observed. There were no significant effects on organ weights, hematology, ophthalmology, clinical signs and mortality. The cholinesterase NOEL is 1 mg/kg/day and the LOEL is 3 mg/kg/day, based on inhibition of serum cholinesterase activity (males) and brain cholinesterase activity (males). The systemic NOEL is 3 mg/kg/day and the LOEL is 12 mg/kg/day based on microscopic effects on the duodenum and slight reductions in body weight, body weight gain and food consumption (Core Guideline; 83-2; MRID 40150121).

f. Developmental Toxicity

Based on the results from the developmental toxicity studies summarized below (effects on the brain) and from the available neurotoxicity studies conducted with fonofos, a developmental neurotoxicity study is required for fonofos (83-6).

Technical fonofos (94%) was tested in a rabbit developmental toxicity study at 0, 0.2, 0.5 or 1.5 mg/kg/day. Eighteen New Zealand White rabbits per group were administered the test material by gavage on gestational days 7 through 19. The maternal NOEL is 1.5 mg/kg/day (HDT). The NOEL for developmental effects is also 1.5 mg/kg/day (HDT). The latter NOEL is borderline because there was a non-statistically significant increase in the number of resorptions/doe in the high-dose group. It was decided that this increase was not toxicologically significant because it was not statistically significant, it is within the historical control range and because the standard deviation for this measurement was so large. The does were considered to be tested at a sufficiently high dose level because in a range-finding study maternal toxicity was observed at 2.0 mg/kg/day and above (1/5 died at this dose level) (Core Minimum; 83-3a; MRID 40150122).

Technical fonofos (95.6%) was tested in a mouse developmental toxicity study at 0, 2, 4, 6 or 8 mg/kg/day. Thirty CD®-1 mice per group were administered the test material by gavage in corn oil on gestational days 6-15 in a dosage volume of 5.0 ml/kg. At 8 mg/kg/day, clinical signs of toxicity (tremors, chromodacryorrhea and dacryorrhea), decreases in body weight gain (85% of controls during dosing period, $p < 0.05$) and slight decreases in food consumption were observed. The maternal NOEL is 6 mg/kg/day and the maternal LEL is 8 mg/kg/day. At 4 mg/kg/day and above, slight dilation of the 4th ventricle in the brain was observed. At 6 mg/kg/day and above, elevations in sternebrae malalignment were observed. The NOEL for developmental effects is 2 mg/kg/day and the LEL is 4 mg/kg/day (Core Minimum; 83-3b; MRID's 00118423 and 42057601).

g. Reproduction

Technical fonofos (99.8-99.9%) was tested in a 3-generation reproduction study in male and female CD albino rats at 0, 10.0 or 31.6 ppm in the diet (0, 0.5 or 1.58 mg/kg/day). The F_0 parents received one-half the respective dose for the first 4 weeks and the F_2 parents received one-half the respective dose for the first week. According to the available data, no effects were observed for either parental systemic toxicity or for reproductive parameters at either dose level. In addition, no effects were observed for pup body weights or viability during lactation. However, deficiencies in the study prevent an

adequate assessment of parental toxicity or reproductive effects. Therefore, an accurate NOEL cannot be estimated (Core Supplementary; 83-4; MRID 00082234).

h. Mutagenicity

Technical fonofos (94.9%) was tested for potential to induce reverse mutations in Salmonella typhimurium, both with and without metabolic activation at the following dose levels: 0.32 1.6, 8.0, 40, 200, 1000 and 5000 ug/plate. The following strains were tested: TA98, TA100, TA1535, TA1537 and TA1538. Fonofos was tested up to levels of cytotoxicity. It did not induce a significant increase in the number of reverse mutations when compared to the vehicle control, DMSO and to the absolute control. The positive controls induced the appropriate responses (Acceptable; 84-2; MRID 41769201).

Technical fonofos (94.9%) was tested for potential to induce chromosomal aberrations in an in vitro assay in human lymphocytes up to cytotoxic levels. The dose levels tested were 10, 50 and 100 ug/ml both with and without metabolic activation. DMSO was used as the vehicle. Fonofos did not induce a significant increase in chromosomal abberations under the conditions of the study. Positive controls (mitomycin C and cyclophosphamide) verified the sensitivity of the assay (Acceptable; 84-2; MRID 41837101).

Technical fonofos (assumed purity of 100%) was tested in an in vivo mouse micronucleus test at 6 and 9.5 mg/kg. The vehicle was corn oil and the positive control was cyclophosphamide in saline. There were no statistically or biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in mice treated with fonofos at either dose level at any of the sampling times investigated, when the data from both sexes were considered separately or when combined (when compared to vehicle control values). The percentage of polychromatic erythrocytes in the treated animals when compared to the controls indicates that there was some indication of cytotoxicity to the bone marrow cells at the dose levels tested. The positive control gave an appropriate positive response. The fonofos treated males had uniformly low values for the percentage of polychromatic erythrocytes, whereas the male vehicle control values were significantly variable. The data indicated that fonofos or a metabolite had induced a cytotoxic effect causing a depression in bone marrow proliferation (Acceptable; 84-2; MRID 41813301).

i. Metabolism

Of the seven metabolism studies in the following paragraphs, the first study was classified as Acceptable (MRID 00090876) and the rest of the studies were classified as Core Supplementary. However, all of the studies when taken together satisfy the regulatory requirements for metabolism studies.

Single oral doses of 2.0, 4.0 or 8.0 mg/kg fonofos, labeled with ^{14}C in the ethyl or phenyl moiety, respectively, were administered to six groups of male rats, two rats/dose group. In addition, three rats (2 males and 1 female) each received single intraperitoneal injections of phenyl-labeled fonofos at 2.0 mg/kg. Three other male rats received ^{14}C-labeled metabolite o-ethylethane-phosphonothioic acid (ETP) at 96.3 mg/kg i.p.. Urine and feces were collected for 48 hours after dosing. Extracts from urine, feces, and bile (analyzed for phenyl-labeled fonofos) were separated by thin layer (TLC) or column techniques, with prior hydrolysis of water-soluble fractions. Quantitation was by liquid scintillation counting. The liver microsomal fraction was obtained from phenobarbital-induced rats and from non-induced rats. Each fraction, together with ethyl-labeled fonofos and ethanol, was incubated in the presence or absence of $NADPH_2$. Analysis was by TLC. From oral administration of fonofos the major metabolites were as follows (average percent radiocarbon administered from the three doses):

Urine	o-ethylethanephosphonothioic acid (ETP)	54.5%
	o-ethylethanephosphonic acid (EOP)	32.7%
	methylphenylsulfone and its phenyl hydrolates (ppm phenyl label)	61.6%
Feces	methylphenyl sulfone ($MPSO_2$ phenyl label)	8.0%
	EOP	3.5%

Metabolites in the bile after i.p. administration consisted mainly of $MPSO_2$ and its hydroxylates (total of 7.9% of administered radiocarbon). Identification of the ethyl-labeled metabolites showed 94.2% of the doses (average) was recovered in the urine and feces within 48 hours, at which time 2.6% of the fonofos (recovered mainly from feces) remained unconverted. Considering recovery data together from the labels in both moieties, a maximum of 87.6% and 13.7% of the orally administered fonofos was eliminated as identified metabolites in the urine and feces, respectively. In vitro results showed that fonofos metabolism at the ethyl moiety requires $NADPH_2$ and is very much enhanced by phenobarbital-induced microsomes (Acceptable; 85-1; MRID 00090876).

It was shown that the metabolites ETP and EDP (along with thiophenol and sulfur are produced by chemical oxidation of fonofos with m-chloroperbenzoic acid (Supplementary; 85-1; MRID 00090877)

A tissue distribution and excretion study of thiophenol (a fonofos metabolite) was conducted. Radiosulfur-labeled thiophenol was administered to young male and female rats at a single oral dosage of either 0.5 or 6 mg/kg. The higher dosage was stated as about 1/10 the LD_{50}. The low dosage was reported as an approximate molar equivalent of the fonofos LD_{50} in rats. Residues of urine, feces, and expired air were collected periodically over 14 days, at which time 15 tissues were analyzed. At 60 hours, the radio-label appeared in the urine and feces (combined) at 75.8% and 94.4% for the high and low dose, respectively (average for males and females). At 14 days, the accounting was 93.0% at the high dose and 100.4% at the low dose, with about 2% in the tissues, mostly in hair and hide. Fat and bone did not contain levels of concern (Supplementary; 85-1; MRID 00090879).

A study of fonofos-C^{14} and fonofos-S^{35} metabolism in the rat shows that the radio-labels are 90% to 97% recovered at 96 hours from either oral or i.p. administration in either sex. Tissue retention at 96 hours averaged 2.3%, mostly in blood, liver, kidney, and intestines. There is a suggestion in the data that male rats may be slightly more efficient than females in eliminating fonofos-S^{35} residues (Supplementary; 85-1; MRID 00090800).

Radio-labeled incubations of induced male rat liver homogenates show that the microsomal metabolism of fonofos, in the presence of $NADPH_2$, produces the potent anticholinergic oxon analog, and the less toxic thiophosphonic acid metabolite, ETP. The oxon metabolite undergoes further microsomal conversion (in absence of $NADPH_2$) to form EOP. Thiophenol is produced in both conversions (Supplementary; 85-1; MRID 00092025).

The rat and corn-plant metabolism of fonofos were compared using TLC techniques. The results were basically quantitative, and the oxygen analog, dyfonox, was the only metabolite identified. The data showed that the major metabolites in corn-plant tissues and rat urine (both water-soluble and benzene-soluble) differ on the basis of chromatographic position (Supplementary; 85-1; MRID's 00043508 and 00090824).

In a study investigating the effect of induction of hepatic microsomal enzymes and biliary excretion, 4 male and 4 female rats were conditioned by oral administration of unlabeled fonofos (0.5 mg/kg/day) for 3 consecutive days. Radiosulfur-labeled fonofos (2.0 mg/kg) was then orally administered to the rats (and

also the unconditioned controls) at various intervals through 96 hours and at 2, 4, 8 and 16 days. Blood and approximately 10 tissues were analyzed for radiolabel at 2, 4, 8 and 16 days. The results showed no significant effect due to possible induction of hepatic enzymes during the 3-day conditioning period. There was essentially no difference in the pattern of excretion; greater than 91% of the label was excreted by both "conditioned" and unconditioned animals at 4 days. A separate trial investigated i.p. administration, with similar results. A biliary excretion study was performed on 3 Long-Evans rats (2 males and 1 female), which were cannulated prior to i.p. dosing with 2 mg/kg of fonofos (in Safflower oil) labeled with C^{14} in the phenyl moiety. Bile collection intervals were 3, 4.5, 22, 28 and 48 hours after dosing. Bile, urine, and feces were assayed by liquid scintillation spectrometry. At 48 hours, 15.3% of the label was excreted in the bile, while 13% of a similar i.p. dose was excreted in the feces of non-cannulated rats. Thus, the data indicate (15.3-13.0)/15.3 = 15% enterohepatic recirculation of labeled material (Supplementary; 85-1; MRID 00090875).

2. Dose Response Assessment

a. Reference Dose

On June 20, 1996, the HED Reference Dose (RfD) Peer Review Committee recommended that the RfD for fonofos be established at 0.002 mg/kg/day. This value was based on the NOEL of 0.2 mg/kg/day (cholinesterase inhibition) from a one-year oral study in beagle dogs (MRID No. 43914601) and an uncertainty factor (UF) of 100. This RfD has not yet been confirmed by the Agency RfD Work Group.

b. Other Toxicological Endpoints (Less Than Lifetime)

The Toxicology Endpoint Selection Committee met on August 6, 1996 to discuss endpoints to be used for acute dietary, short term, intermediate term and chronic occupational or residential exposures.

Acute Dietary

For the acute dietary endpoint, the developmental rabbit study, including the range-finding study was selected with the acute neurotoxicity study in the rat as the supporting study. The observed effect in the rabbit study was maternal deaths. **2.0 mg/kg/day was selected as the NOEL for acute exposure.** One death from 5 rabbits was observed at this dose level, but only after 5 exposures. The supporting acute neurotoxicity study had a NOEL of 4 mg/kg and a LOEL of 7 mg/kg, at which clinical signs of toxicity were observed by 6 hours and resolved by 24 hours.

Short Term Occupational or Residential Exposure (1 to 7 Days)

For short term exposure, 3 co-critical studies were selected for determination of an endpoint. The 3 studies are a 98-day feeding and a chronic feeding study in the dog and the developmental study in the rabbit with the range-finding study. **The NOEL selected was 1.5 mg/kg/day from the 98-day dog study, a dose above which effects were observed within the first few days of each of the co-critical studies.** At 2.0 mg/kg/day in the rabbit study, death was observed in one animal by the 5th dosing day. At 1.75 and 2.0 mg/kg/day in the chronic dog study, clinical signs of toxicity were observed in several dogs on day 3. The effects seen at 1.5 mg/kg/day and at 0.4 mg/kg/day, the next lower dose level in the 98-day dog study were observed later in the study and were not considered relevant to short term exposure. For dermal exposures an absorption factor of 100% will be used for risk assessment until a dermal absorption study becomes available.

Intermediate Term Occupational or Residential Exposure (1 Week to Several Months)

For intermediate term exposure, the NOEL of 0.75 mg/kg/day from the 90-day neurotoxicity study in the rat was selected. The LEL was 2.5 mg/kg/day based on inhibition of erythrocyte, plasma and brain cholinesterases. The decreases in plasma and erythrocyte cholinesterases started at week 4. At the highest dose level, 6.25/7.5 mg/kg/day there were decreases in motor activity starting at week 5 and clinical signs of toxicity starting at week 9. For dermal exposures an absorption factor of 100% will be used for risk assessment until a dermal absorption study becomes available.

Chronic Occupational or Residential Exposure

For chronic exposure, the NOEL of 0.2 mg/kg/day from the chronic feeding study in the dog was selected. The LEL was 1.0 mg/kg/day based on increases in alkaline phosphatase levels and decreases in erythrocyte and plasma cholinesterase levels. At 1.75 mg/kg/day, there were clinical signs of toxicity on day 3, changes in clinical chemistry, increases in absolute liver weights and decreases in all 3 cholinesterase values. For dermal exposures an absorption factor of 100% will be used for risk assessment until a dermal absorption study becomes available.

Inhalation Exposure (Any Time Period)

No long term inhalation studies are available. Based on the LD_{50} of 50 µg/L in males and 17.9 µg/L in females, fonofos is placed in toxicity category I. Therefore, any risk assessment should be inclusive of the inhalation (100%) plus the dermal (100%) exposures.

c. Carcinogenicity Assessment

On August 12, 1993, the HED Reference Dose (RfD) Peer Review Committee recommended that fonofos be classified as a Group E carcinogen (no evidence of carcinogenic potential in long-term studies in rats and mice).

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Chemical:	**Phosphonodithioic acid, ethyl-, O-ethyl**
PC Code:	**041701**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/11/96**
File ID:	**DPD229323**
Accession Number:	**412-02-0005**

HED Records Reference Center
10/25/2001